DRAFT: August 31, 1994

DECISION DOCUMENT
TSCA SECTION 5(H)(4) EXEMPTION FOR
ASPERGILLUS _NIGER_

I. SUMMARY

Aspergillus _niger_ is an asexual fungus commonly found degrading organic matter in nature. _A_. _niger_ strains are significant organisms in the fermentation industry for the production of citric acid and several enzymes and generally have a history of safe use. Members of the species are ubiquitous in nature and most of the human population is frequently exposed to them. The hazards associated with this species appear to be both strain and culture environment specific. _A_. _niger_ strains have been found to produce mycotoxins, especially malformin C. There is some difficulty properly identifying new isolates from the field. Strains can be tested for the production of specific mycotoxins associated with the species. The potential risks from use of _A_. _niger_ in fermentation facilities are low.

II. BACKGROUND

A. Introduction

EPA recognizes that some microorganisms present a low risk when used under specific conditions at general commercial use. Therefore, EPA is proposing expedited regulatory processes for certain microorganisms under these specific conditions at the general commercial use stage. Microorganism uses that would be exempt meet criteria addressing: (1) performance based standards for minimizing the numbers of microorganisms emitted from the manufacturing facility; (2) the introduced genetic material; and (3) the recipient microorganism. Microorganisms that qualify for these exemptions, termed Tier I and Tier II, must meet a standard of no unreasonable risk in the exempted use.

To evaluate the potential for unreasonable risk to human health or the environment in developing these exemptions, EPA focuses primarily on the characteristics of the recipient microorganisms. If the recipient is shown to have little or no potential for adverse effects, introduced genetic material meeting the specified criteria would not likely significantly increase potential for adverse effects. As further assurance that risks would be low, EPA is also specifying procedures for minimizing numbers of organisms emitted from the facility. When balanced against resource savings for society and expected product benefits, these exemptions will not present unreasonable risks.

B. Criteria for Minimizing Release from Manufacturing Facilities

The standards prescribed for the Tier I exemption require the following: (1) the structure(s) be designed and operated to contain the microorganism, (2) access to the structure should be limited to essential personnel, (3) inactivation procedures shown to be effective in reducing the number of viable microorganisms in liquid and solid wastes should be followed prior to disposal of the wastes, (4) features to reduce microbial concentrations in aerosols and exhaust gases released from the structure should be in place, and (5) general worker hygiene and protection practices should be followed.

1. Definition of structure. EPA considers the term "structure" to refer to the building or vessel which effectively surrounds and encloses the microorganism. Vessels may have a variety of forms, e.g., cubic, ovoid, cylindrical, or spherical, and may be the fermentation vessel proper or part of the downstream product separation and purification line. All would perform the function of enclosing the microorganism. In general, the material used in the construction of such structure(s) would be impermeable, resistant to corrosion and easy to clean/sterilize. Seams, joints, fittings, associated process piping, fasteners and other similar elements would be sealed.

2. Standards to minimize microbial release. EPA is proposing, for several reasons, a somewhat cautious approach in prescribing standards for minimizing the number of microorganisms emitted through the disposal of waste and the venting of gases. First, a wide range of behaviors can be displayed by microorganisms modified consistent with EPA's standards for the introduced genetic material. Second, EPA will not conduct any review whatsoever for Tier I exemptions. EPA believes the requirement to minimize emissions will provide a measure of risk reduction necessary for making a finding of no unreasonable risk. Taken together, EPA's standards ensure that the number of microorganisms emitted from the structure is minimized.

EPA's proposed standards for minimizing emission specify that liquid and solid waste containing the microorganisms be treated to give a validated decrease in viable microbial populations so that at least 99.9999 percent of the organisms resulting from the fermentation will be killed. Since the bacteria used in fermentation processes are usually debilitated, either intentionally or through acclimation to industrial fermentation, the small fraction of microorganisms remaining viable after inactivation treatments will likely have a reduced ability to survive during disposal or in the environment. Moreover, industrial companies, in an attempt to keep their proprietary microorganisms from competitors and to reduce the microbial numbers to those permitted by local sanitation authorities, modify the microorganisms to increase the ability of their microorganisms to survive and perform their assigned tasks in the fermentor but decrease their ability to survive in the environment external to the fermentor.

EPA requirements also address microorganisms in the exhaust from the fermentor and along the production line. To address exhaust from fermentors, EPA is proposing that the number of microorganisms in fermentor gases be reduced by at least two logs prior to the gases being exhausted from the fermentor. EPA selected this number based on an estimate of the numbers of microorganisms likely to be in the exhaust from an uncontrolled fermentor and common industry practice. Moreover, microorganisms that are physiologically acclimated to the growth conditions within the fermentor are likely to be compromised in their ability to survive aerosolization. EPA anticipates, therefore, that few microorganisms will survive the stresses of aerosolization associated with being exhausted in a gas from the fermentor. The provision requiring reduction of microorganisms in fermentor exhaust gases contributes to minimizing the number of viable microorganisms emitted from the facility.

EPA is also proposing that the requirements specify that other systems be in place to control dissemination of microorganisms by other routes. This would include programs to control pests such as insects or rats, since these might serve as vectors for carrying microorganisms out of the fermentation facilities.

3. Worker protection. The requirement to minimize microbial emissions, in conjunction with the requirement for general worker safety and hygiene procedures, also affords a measure of protection for workers. Potential effects on workers that exist with microorganisms in general (e.g., allergenicity) will be present with the microorganisms qualifying for this exemption. As with other substances that humans may react to (e.g., pollen, chemicals, dust), the type and degree of allergenic response is determined by the biology of the exposed individual. It is unlikely that a microorganism modified in keeping with EPA's specifications for the introduced genetic material would induce a heightened response. The general worker hygiene procedures specified by EPA should protect most individuals from the allergenic responses associated with

microorganisms exhausted from fermentors and/or other substances emitted along the production line. The EPA requirement that entry be limited to essential personnel also addresses this consideration by reducing to a minimum the number of individuals exposed.

4. Effect of containment criteria. As further assurance that risks would be low, EPA is specifying procedures for minimizing numbers of organisms emitted from the facility for the Tier I exemption. EPA is not specifying standards for minimizing the number of microorganisms emitted from the facility for microorganisms qualifying for Tier II exemption. Rather, the Agency requests that submitters utilize as guidance the standards set forth for Tier I procedures. The procedures proposed by the submitter in a Tier II exemption request will be reviewed by the Agency. EPA will have the opportunity to evaluate whether the procedures the submitter intends to implement for reducing the number of organisms emitted from the facility are appropriate for that microorganism.

C. Introduced Genetic Material Criteria

In order to qualify for either Tier I or Tier II exemption, any introduced genetic material must be limited in size, well characterized, free of certain nucleotide sequences, and poorly mobilizable.

1. Limited in size. Introduced genetic material must be limited in size to consist only of the following: (1) the structural gene(s) of interest; (2) the regulatory sequences permitting the expression of solely the gene(s) of interest; (3) the associated nucleotide sequences needed to move genetic material, including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites; (4) the nucleotide sequences needed for vector transfer; and (5) the nucleotide sequences needed for vector maintenance.

The limited in size criterion reduces risk by excluding the introduction into a recipient of extraneous and potentially uncharacterized genetic material. The requirement that the regulatory sequences permit the expression solely of the structural gene(s) of interest reduces risk by preventing expression of genes downstream of the inserted genetic material. The limitation on the vector sequences that are components of the introduced genetic material prevents the introduction of novel traits beyond those associated with the gene(s) of interest. The overall result of the limited in size criterion is improved ability to predict the behavior of the resulting microorganism.

2. Well characterized. For introduced genetic material, well characterized means that the following have been determined: (1) the function of all of the products expressed from the structural gene(s); (2) the function of sequences that participate in the regulation of expression of the structural gene(s); and (3) the presence or absence of associated nucleotide sequences.

Well characterized includes knowledge of the function of the introduced sequences and the phenotypic expression associated with the introduced genetic material. Genetic material which has been examined at the restriction map or sequence level, but for which a function or phenotypic trait has not yet been ascribed, is not considered well characterized. Well characterized would include knowing whether multiple reading frames exist within the operon. This relates to whether more than one biological product might be encoded by a single sequence, and addresses the possibility that a modified microorganism could display unpredicted behavior should such multiple reading frames exist and their action not be anticipated.

3. Free of certain sequences. In addition to improving the ability to predict the behavior of the modified microorganism, the well characterized requirement ensures that segments encoding for either part or the whole of the toxins listed in the proposed regulatory text for the TSCA biotechnology rule would not inadvertently be introduced into the recipient microorganism.

These toxins are polypeptides of relatively high potency. Other types of toxins (e.g., modified amino acids, heterocyclic compounds, complex polysaccharides, glycoproteins, and peptides) are not listed for two reasons. First, their toxicity falls within the range of moderate to low. Second, these types of toxins generally arise from the activity of a number of genes in several metabolic pathways (multigenic).

In order for a microorganism to produce toxins of multigenic origin, a large number of different sequences would have to be introduced and appropriately expressed. It is unlikely that all of the genetic material necessary for metabolizing multigenic toxins would be inadvertently introduced into a recipient microorganism when requirements that the genetic material be limited in size and well characterized are followed.

Similarly, other properties that might present risk concerns result from the interactive expression of a large number of genes. For example, pathogenic behavior is the result of a large number of genes being appropriately expressed. Because of the complex nature of behaviors such as pathogenicity, the probability is low that an insert consisting of well characterized, limited in size genetic material could transform the microorganisms proposed for exemption into microorganisms which display pathogenic behavior.

4. Poorly mobilizable. Poorly mobilizable means the ability of the introduced genetic material to be transferred and mobilized is inactivated, with a resulting frequency of transfer of less than 10^{-8} transfer events per recipient. The requirement that the introduced genetic material be poorly mobilizable reduces potential for transfer of introduced genetic sequences to other microorganisms in the environment. Such transfers would occur through the interaction of the introduced microorganism with indigenous microorganisms through conjugation, transduction, or transformation. Through such transfers, the introduced genetic material could be transferred to and propagated within different populations of microorganisms, including microorganisms which may never previously have been exposed to this genetic material. It is not possible to predict how the behavior of these potential recipient microorganisms will be affected after uptake and expression of the genetic material.

Since EPA is not limiting the type of organism that can serve as the source for the introduced genetic material, some limitation is placed on the ability of the introduced genetic material to be transferred. This limitation mitigates risk by significantly reducing the probability that the introduced genetic material would be transferred to and expressed by other microorganisms.

The 10^{-8} frequency is attainable given current techniques. Plasmids with transfer rates of 10^{-8} exist or are easily constructed. Some of the plasmids most commonly employed as vectors in genetic engineering (e.g., pBR325, pBR322) have mobilization/transfer frequencies of 10^{-8} or less.

The criteria set for "poorly mobilizable" for transduction and transformation should not prevent most microorganisms from meeting the exemption criteria, since the majority of transfer frequencies reported for transduction and natural transformation are less than 10^{-8}. Higher frequencies are likely only if the introduced genetic material has been altered or selected to enhance frequency.

Fungal gene transfer has also been considered in development of the poorly mobilizable criterion. Although mobile genetic elements such as transposons, plasmids and double stranded RNA exist in fungi and can be readily transferred, this transfer usually is only possible between members of the same species during anastomosis, a process specific to fungi. Since anastomosis only occurs between members of the same species, the introduced genetic material would not be transferred to distantly related fungi as may occur with bacteria.

5. Effect of introduced genetic material criteria. The requirements placed on the introduced genetic material, in concert with the level of safety associated with Aspergillus niger, ensure that the resulting microorganisms present low or negligible risk. The probability is low that the insertion of genetic material meeting EPA's criteria into strains of A. niger will change their behavior so that they would acquire the potential for causing adverse effects. Risks would be mitigated by the four criteria placed on the introduced genetic material, the relative safety of A. niger, and the inactivation criteria specified for the Tier I exemption. In the case of Tier II exemption, risks would be mitigated in light of the four criteria placed on introduced genetic material, the relative safety of A. niger, and EPA's review of the conditions selected.

D. Recipient Microorganism Criteria

Six criteria were used by EPA to determine eligibility of recipient microorganisms for the tiered exemption. Microorganisms which EPA finds meet these criteria are listed as eligible recipients. The first criteria would require that it be possible to clearly identify and classify the microorganism. Available genotypic and phenotypic information should allow the microorganism to be assigned without confusion to an existing taxon which is easily recognized. Second, information should be available to evaluate the relationship of the microorganism to any other closely related microorganisms which have a potential for adverse effects on human health or the environment. Third, there should be a history of commercial use for the microorganism. Fourth, the commercial uses should indicate that the microorganism products might be subject to TSCA jurisdiction. Fifth, studies are available which indicate the potential for the microorganism to cause adverse effects on human health and the environment. Sixth, studies are available which indicate the survival characteristics of the microorganism in the environment.

After each microorganism was reviewed using the six evaluation criteria, a decision was made as to whether to place the microorganism on the list. The Agency's specific determination for Aspergillus niger is discussed in the next unit.

III. EVALUATION OF ASPERGILLUS NIGER

A. History of Use

1. History of safe commercial use. The primary uses of A. niger are for the production of enzymes and organic acids by fermentation. The history of safe use for A. niger comes primarily from its use in the food industry for the production of many enzymes and organic acids, such as citric acid. A. niger is considered a Class 1 Containment Agent under the NIH Guidelines for Research Involving Recombinant DNA Molecules. In Europe, Aspergillus species are considered category 2 under the European Federation of Biotechnology guidelines and category 1 under the OECD containment scale.

2. Products Subject to TSCA jurisdiction. While EPA has not yet received a submission for a strain of A. niger, some of the future uses of enzymes derived from A. niger could be subject to TSCA. A. niger has some uses as the organism itself, in addition to its products of fermentation. For example, due to its ease of visualization and resistance to several anti-fungal agents, A. niger is used to test the efficacy of preservative treatments (Jong and Gantt, 1987). In addition, A. niger has been shown to be exquisitely sensitive to micronutrient deficiencies prompting the use of A. niger strains for soil testing (Raper and Fennell, 1965). There is also interest in using this fungus to perform certain enzymatic reactions that are very difficult to accomplish by strictly chemical means, such as specific additions to steroids and other complex rings (Jong and Gantt, 1987).

B. Identification of Microorganism

1. Classification. The taxonomy of Aspergillus is primarily based on morphological features, rather than physiological, biochemical features and genetic characteristics. Morphology provides a reasonable means of classification and assignment within the *A. niger* group.

2. Related species of concern. Some members of the genus Aspergillus produce potent mycotoxins. The A. flavus group produce aflatoxins which are acutely toxic to mammals and are classified as probable human carcinogens. However, A. niger is not a member of the A. flavus group, and proper identification should avoid confusion between the two species.

C. Risk Summary

1. Studies regarding potential for adverse effects. Aspergillus niger is an opportunistic pathogen capable of colonizing spaces in the body. From these spaces it may or may not invade neighboring tissues. Invasions of neighboring tissues appears only to occur with immunocompromised patients. Since all humans are exposed to aspergilli species, but disease is rare, the physiological state of the exposed individual must be paramount. A. niger produces mycotoxins, and can induce allergic responses such as occupational asthma. The reports of occupational asthma associated with use of A. niger appear to be of isolated incidents involving a few specific strains. A. niger produces the mycotoxins malformins A and C which have been shown to be toxic to mammals. Toxicity was determined by intraperitoneal injection, a route not considered to be relevant to environmental exposures. The malformins are apparently much less toxic when ingested.

Under some conditions, A. niger appears to be a pathogen to peanuts. Because it can grow on a variety of substrates, A. niger can cause spoilage of bakery, fruit and vegetable products and can damage surface layers of wood, raw cotton fibers and other materials.

2. Studies regarding survival in the environment. A. niger is ubiquitous in nature. It is geographically widely distributed and can colonize a wide variety of substrates. A. niger is commonly found as a saprophyte growing on decaying vegetation.

IV. BENEFITS SUMMARY

Substantial benefits are associated with this proposed exemption. Aspergillus niger is already widely employed in general commercial uses, some of which are subject to TSCA reporting. The Agency believes this exemption will result in resource savings both to EPA and industry without compromising the level of risk management afforded by the full 90 day review. In addition to assessing the risk of A. niger, EPA has developed criteria limiting the potential for transfer of and expression of toxin sequences, and the conditions of use specified in the exemption are met (Tier I) or will be reviewed by EPA to ensure adequate risk reduction (Tier II). EPA requirements for minimizing numbers of viable microorganisms emitted are within standard operating procedures for the industry, and both the procedures and the structures specified in the exemption are the type industry uses to protect their products from contamination.

The exemption will result in reduced reporting costs and a decrease in delay associated with reporting requirements. The savings in Agency resources can be directed to reviewing activities and microorganisms which present greater uncertainty. This exemption should also facilitate development and manufacturing of new products and the accumulation of useful information.

V. RECOMMENDATION AND RATIONALE

A. Recommendation: Aspergillus niger is recommended for a TSCA section 5(h)(4) tiered exemption.

B. Rationale

1. Risks from use of the recipient microorganism A. niger are low. Humans are continually exposed to A. niger spores and vegetative forms on foodstuffs and in the air. While the greatest concerns for any adverse effects would be for workers who could be exposed to much greater spore concentrations than the general public, the vast majority of A. niger strains used for industrial fermentation have a history of safe use. Cases involving mycotoxin production or allergic responses by workers exposed to A. niger appear to be associated with a limited number of strains. Given that the probability of colonization of immunocompetent workers is quite small and that A. niger is ubiquitous in the environment, use of A. niger strains in commercial fermentation facilities should present low risks to human health and the environment.

2. Risks from use of strains of A. niger which are eligible for the TSCA section 5(h)(4) exemption present no unreasonable risk. A concern has been expressed that Aspergillus strains could be mistakenly identified as A. niger when they were in fact one of the other Aspergillus species which produce more potent mycotoxins, such as aflatoxins. However, as part of their eligibility for this TSCA section 5(h)(4) exemption, companies are required to certify that they are using A. niger. It is therefore expected that companies will have information in their files which documents the correct identification of their strains. Additionally, it is expected that companies will choose well-characterized industrial strains for further development through genetic modification. These expectations in combination with the use of Good Laboratory Practices should ensure the use of the correct species.

While production of certain mycotoxins has been associated with strains of A. niger, companies have been using strains of A.

niger to produce a variety of products for many years without reports of toxic effects of workers. The limited in size constraints as well as the restriction on vertebrate toxins imposed on introduced DNA by the criteria for the section 5(h)(4) exemption should reduce the likelihood of increased production or exposure to malformins A and C, the two most potent mycotoxins potentially produced by A. niger strains.

Because the recipient microorganism was found to have little potential for adverse effects, introduced genetic material meeting the specified criteria would not likely significantly increase potential for adverse effects. As further assurance that risks would be low, EPA is specifying procedures for minimizing numbers of organisms emitted from the facility for the Tier I exemption and will be reviewing the conditions selected for the Tier II exemption. When balanced against resource savings for society and expected product benefits, this exemption will not present unreasonable risks.

Attachment 1:

INTEGRATED RISK ASSESSMENT FOR

Aspergillus niger

I. INTRODUCTION

Aspergillus niger is a member of the genus *Aspergillus* which includes a set of fungi that are generally considered asexual, although perfect forms (forms that reproduce sexually) have been found. Aspergilli are ubiquitous in nature. They are geographically widely distributed, and have been observed in a broad range of habitats, because they can colonize a wide variety of substrates. *A. niger* is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles, and other decaying vegetation. The spores are widespread, and are often associated with organic materials and soil.

History of Commercial Use and Products Subject to TSCA Jurisdiction

The primary uses of *A. niger* are for the production of enzymes and organic acids by fermentation. While the foods, for which some of the enzymes may be used in preparation, are not subject to TSCA, these enzymes may have multiple uses, many of which are not regulated except under TSCA. Fermentations to produce these enzymes may be carried out in vessels as large as 100,000 liters (Finkelstein et al., 1989). *A. niger* is also used to produce organic acids such as citric acid and gluconic acid.

The history of safe use for *A. niger* comes primarily from its use in the food industry for the production of many enzymes such as a-amylase, amyloglucosidase, cellulases, lactase, invertase, pectinases, and acid proteases (Bennett, 1985a; Ward, 1989). In addition, the annual production of citric acid by fermentation is now approximately 350,000 tons, using either *A. niger* or *Candida* yeast as the producing organisms. Citric acid fermentation using *A. niger* is carried out commercially in both surface culture and in submerged processes (Berry et al., 1977; Kubicek and Rohr, 1986; Ward, 1989).

A. niger has some uses as the organism itself, in addition to its products of fermentation. For example, due to its ease of visualization and resistance to several anti-fungal agents, *A. niger* is used to test the efficacy of preservative treatments (Jong and Gantt, 1987). In addition, *A. niger* has been shown to be exquisitely sensitive to micronutrient deficiencies prompting the use of *A. niger* strains for soil testing (Raper and Fennell, 1965). There is also interest in using this fungus to perform certain enzymatic reactions that are very difficult to accomplish by strictly chemical means, such as specific additions to steroids and other complex rings (Jong and Gantt, 1987).

II. IDENTIFICATION AND TAXONOMY

As is the case of many fungi, the taxonomy of *Aspergillus* is primarily based on morphological features, rather than the physiological, biochemical features and genetic characteristics often used to classify bacteria. The genus *Aspergillus* is usually defined as asexual saprophytic fungi that produce large black or brown conidia by phialides that are arranged in a globose head radiating from a vesicle or spherical conidiophore. This definition leads to inclusion of a complex assortment of organisms within the taxon. This is illustrated by the 132 species arranged in 18 groups by Raper and Fennell (1965) due to overlapping morphological or physiological characteristics. *Aspergillus niger* is both a species and a group within the genus *Aspergillus*.

The morphological approach to taxonomy has led to the existence of several synonyms for the genus *Aspergillus*. They are: *Alliospora* Pim; *Aspergillonsis* Spegazzini; *Cladaspergillus* Ritg; *Cladosparum* Yuill and Yuill; *Euaspergilus* Ludwig;

Gutturomyces Rivolta; *Raperia* Subramaniam and Grove; *Sceptromyces* Corda; *Spermatoloncha* Spegazzini; *Sphaeromyces* Montagne; *Sterigmatocystis* Cramer; and *Stilbothamnium* Hennings (Bennett, 1985).

A. Definition of the *Aspergillus niger* Group

Raper and Fennell (1965) designated 15 species as comprising the *Aspergillus niger* group, which includes all of the Aspergilli with black conidia. There have been suggestions to subdivide further (Al-Musallam, 1980), but currently the concept of retention of the *A. niger* group based on black conidia seems dominant (Kusters-van Someren et al., 1990).

More sophisticated means of treating the classification of fungi have been attempted. Mullaney and Klich (1990) reviewed the molecular biological techniques for taxonomic classification studies of *Aspergillus* and *Penicillium* which include G + C molar percentage, DNA:DNA complementarity (measuring rate and extent of reassociation of single stranded DNA from two isolates), ribosomal RNA sequence comparison, and restriction fragment length polymorphism. One study of restriction digests of mitochondrial DNA indicated that all the *Aspergillus* groups examined are related. However, *A. niger* and *A. awamori*, both in the niger group, appear less related than would be expected for members in the same group (Kozlowski and Stepien, 1982). Work in the area of DNA homology and relatedness among the black aspergilli is ongoing at the USDA Northern Regional Research Laboratory in Peoria, IL (Peterson, 1991). More exhaustive use of these and related techniques may give a clearer taxonomic system which will permit better separation of its members.

B. *A. niger* Species

While morphology provides a reasonable means of classification and assignment within the *A. niger* group, it is not a reliable means for identifying a given isolate from the field. The major distinction currently separating *A. niger* from the other species of *Aspergillus* is the production of carbon black or very dark brown spores from biseriate phialides (Raper and Fennell, 1965). Other features include the smooth and generally colorless conidiophores and spores that are #5 µm, globose and have conspicuous ridges or spines not arranged in rows. *A. niger* isolates grow slowly on Czapek agar (Raper and Fennell, 1965). These physical characters such as spore color and rate of growth on a defined media are subject to change, especially under extended pure culture or selection and mutation. Though *A. niger* is relatively stable to spontaneous mutation compared to other aspergilli, variation in morphology may still be a problem with some strains (Raper and Fennell, 1965). Thus this species may be misidentified with other *Aspergillus* spp.

C. Potential Nomenclature Problems

Nomenclature problems of the genus *Aspergillus* arise from their pleomorphic life cycle. The newer findings show that this group of fungi has both a perfect (teleomorphic) and an imperfect (anamorphic) state. The International Code of Botanical Nomenclature provides a system of 76 mandatory rules (Articles), and also Recommendations, to promote nomenclature stability (Hawksworth, 1990). In a retrospective revision of the rules concerning fungi with pleomorphic life cycles, Art. 59, adopted by the 1981 International Botanic Congress (Voss et al., 1983), the decision was reached that "even if a species name was proposed under an anamorphic generic name, if the description and the type included the sexual ascosporic stage, then the name had to be applied to the teleomorph and was no longer available to the anamorph, the conidial state" (Hawksworth, 1990). Article 14 of the Code provides for conservation procedures to avoid disadvantageous changes in well-known family and generic names due to strict application of the code.

To avoid confusion, for economic or public health reasons taxonomists make exceptions to their rules. Thus, conservation of well-known names was also allowed for "species of major economic importance" (Art. 14.2) at the 1981 International Botanic Congress (Voss et al., 1983). Frisvad et al. (1990) pointed out that of the two obviously threatened names in the taxonomy of *Aspergillus*, *A. niger* van Tieghem is one of great importance. With this in mind Hawksworth (1990) recommended that the *Aspergilli* be included in a pilot study for the "List of Names in Current Use" initiative that could lead to formal adoption if sanctioned by the International Commission on the Taxonomy of Fungi.

If the rules for naming are rigorously applied, *A. niger* might disappear as a legitimate name, causing great commercial confusion. Al-Musallam (1980) stated that there are two species described in the last century, *A. phoenicus* (Corda) Thom (1840) and *A. ficuum* (Reichardt) Hennings (1867) accepted as valid species by Thom and Raper (1945) and again by Raper and Fennel (1965) that are the same as *A. niger*, or that is a variety of one of them. However, Frisvad et al. (1990) believe that a clear case exists for conserving the name *A. niger,* because *A. niger* is "the source of commercial production of citric acid and other organic acids around the world, and clearly of major economic importance." The earlier names have been used only rarely in modern publications. Thus, possible revision of the taxonomy of *Aspergillus* does not seem to include replacement of *A. niger* for the foreseeable future.

D. Conclusions on Taxonomy and Identification

Thus, while the name *A. niger* seems secure for now, the organisms to which it applies still represent a complex amalgam of morphologically related isolates. Those collections that take care to control conditions of culture and apply rigorous methods during identification should be able properly identify strains as belonging to this species. However, that does not guarantee that all strains properly called *A. niger* will share most physiological properties. The ones most likely to be well defined are those having long histories in culture, especially commercial culture, where the knowledge of these physiological properties is important to their maintenance. Since some features of concern for hazard may not be related to the morphological features used for classification, information on the physiology and biochemistry of *A. niger* strains maintained in culture, as well as their morphology, is useful for confirmation of identity.

E. Related Species of Concern

The taxonomy of *Aspergillus* has public health implications due to the production of potent mycotoxins by members of this genus. Most notable of these is the association of aflatoxins with members of the *A. flavus* group (Bennett, J.W. (1985b), Semeniuk et al., (1971)). *A. niger* is not a member of that group, generally being distinguishable by color and structure of the conidial head (Raper and Fennel (1965)). Though proper separation among aspergilli requires a trained mycologist and care for proper culture conditions, when this is accomplished there should not be confusion between *A. niger* strains and members of the *A. flavus* group.

III. HAZARD ASSESSMENT

A. Human Health Hazards

Three categories of human health effects are examined in this risk assessment of *A. niger*: 1) colonization of spaces in the body and potential invasion of neighboring tissues; 2) production of mycotoxins; and 3) induction of allergic responses such as occupational asthma.

1. Colonization and Pathogenicity

The growth of the fungus *Aspergillus* in human tissue or within air-containing spaces of the body, such as bronchus or pulmonary cavity, is termed aspergillosis (Bennett, 1979a). Exposure to *Aspergillus* must be nearly universal but disease is rare. The physiological condition of the exposed individual thus appears to be of paramount importance. Patients exhibiting aspergillosis are generally immunocompromised, and thus susceptible to otherwise common and usually harmless microorganisms. Factors that may lead to immunosuppression include an underlying debilitating disease (e.g., chronic granulomatous diseases of childhood), chemotherapy, and the use of supraphysiological doses of adrenal corticosteroids (Bennett, 1980).

Pulmonary aspergillosis is the most common clinical manifestation of aspergillosis. The most common symptoms of pulmonary aspergillosis are a chronic productive cough and hemoptysis (coughing up blood). According to a standard medical textbook, "*Aspergillus* can colonize ectatic bronchi, cysts, or cavities in the lung. Colonization is usually a sequel of a chronic inflammatory process, such as tuberculosis, bronchiectasis, histoplasmosis, or sarcoidosis. A ball of hyphae may form within an air-containing space, particularly in the upper lobes, and is termed an aspergilloma. The fungus rarely invades the wall of the cavity, cyst, or bronchus in such patients" (Bennett, 1979a). It is not clear what role *Aspergillus* plays in noninvasive lung disease. Plugs of hyphae may obstruct bronchi. Perhaps allergic or toxic reaction to *Aspergillus* antigens could cause bronchial constriction and damage (Bennett, 1980).

Although *Aspergillus fumigatus* is the usual cause of aspergillosis (Bennett, 1979b), there have been several recent case reports of pulmonary aspergillosis caused by *A. niger*. For example, Kierownik (1990) described a 66-year-old man who was admitted to the hospital with pulmonary lesions and cavitation of his lung. Fungi were cultured and the sputum contained fungal forms typical for *A. niger* complicating a pulmonary abscess in the course of a pneumonia. Korzeniowska-Kosela et al. (1990) also describe a pulmonary aspergilloma caused by *A. niger*. Medina et al. (1989) reported on cases of bilateral maxillary sinusitis and a right pansinusitis. In a case described by Louthrenoo et al. (1990), an amputation of the right foot had to be performed on a malnourished 70-year-old man who presented with a painful black "gangrenous appearing" mass on the right foot. Tissue samples showed not only branching hyphae, but dark pigmented fungal fruiting heads with double sterigmata.

Aspergillus niger was identified.

Although *Aspergillus niger* is regarded as an opportunistic pathogen (Padhye, 1982; Walsh and Pizzo, 1988), it can cause otomycosis in healthy, noncompromised persons who have no underlying disease (Austwick, 1965). Otomycosis is the name given to the growth of *Aspergillus*, often *A. niger*, on ceruman and desquamated debris in the external auditory canal. The condition is benign. Of 159 suspected cases of otomycosis in Nigeria, 36 were specifically confirmed on the basis of demonstrating microscopically fungal structures in epithelial debris plugs and a positive culture (Gugnani et al., 1989). Another 31 cases gave positive cultures but were negative microscopically, and thus were considered of doubtful fungal pathology. Again, *A. niger* was predominant.

Both the severity of aspergillosis and the patient's prognosis are dependent on the physiologic status of the patient. Invasion of lung tissue in aspergillosis is almost entirely confined to immunosuppressive patients (Bennett, 1980). Roughly 90 percent of invasive pulmonary case patients will have two of these three conditions: severe immunosuppression (less than 500 granulocytes per cubic millimeter of peripheral blood). supraphysiological doses of adrenal corticosteroids, and a history of taking cytotoxic drugs such as azathioprine (Bennett, 1980). In addition, the type of disease produced affects the patient's chances for recovery. For example, simple colonization is treatable, but if the simple colonization becomes chronic or invades neighboring tissues, the infection becomes more difficult to treat (McGinnis, 1980). Surgical excision has been used successfully to treat invasive aspergillosis of the paranasal sinus as well as noninvasive sinus colonization. Intravenous amphotericin B has resulted in arrest or cure of invasive aspergillosis when immunosuppression is not severe (Bennett, 1980). Pleural aspergillosis often responds well to surgical drainage alone (Bennett, 1979b).

2. Allergic Reactions to *Aspergillus niger*

Allergens produced by *A. niger* can produce allergic disease in humans. When inhaled, *A. niger* can cause hypersensitivity reactions such as asthma and allergic alveolitis (Edwards and Al-Zubaidy, 1977). However, only a few instances of asthma induced by *A. niger* have been reported. One such instance involved a manufacturing plant in which a specially selected strain of *A. niger* was being used to ferment molasses to produce citric acid. Both stirred tank and surface methods were being used. Eighteen workers were diagnosed as having occupational asthma; half had IgE antibody to *A. niger* based on skin and RAST tests. As determined by RAST inhibition experiments using a commercial extract of *A. niger*, the antigen that caused the sensitization appeared to be peculiar to the *A. niger* strain used for the fermentation (Topping et al., 1985).

In studies on 30 of 83 patients who showed symptoms of bronchial asthma, it was found that skin hypersensitivity to *Aspergillus* antigens with a high serum IgE to these antigens is indicative of *Aspergillus* sensitivity. In addition, levels of IgE protein and IgE antibodies specific for eight different allergenic extracts (including *Aspergillus*) were measured in the serum of persons infected with human immunodeficiency virus (HIV) and HIV negative subjects belonging to the same high risk group. Levels of IgE protein and antibodies were found to be definitively higher in the HIV infected patients (Maggi et al., 1989).

Massive inhalation of *Aspergillus* spores by normal persons can lead to an acute, diffuse, self-limiting pneumonitis. Spontaneous recovery taking several weeks is the usual course (Bennett, 1980). For example, Dykewicz et al. (1988) described the case of a 28-year-old man who developed fevers, cough, shortness of breath and other symptoms several hours after cutting live oak and maple trees. Fungal cultures of the wood chips yielded *A. niger* along with other *Aspergillus* species, three species of *Penicillium*, *Paecilomyces sp*., and *Rhizopus sp*. Several immunological techniques were used to show that the *Penicillium sp*. were probably responsible. Reports such as this illustrate that *A. niger*, while implicated by its isolation in association with some cases, is not necessarily the causative agent.

3. Toxin Production by *A. niger*

Aspergillus niger can produce a variety of fungal metabolites, termed mycotoxins, depending upon growth conditions and the strain of the organisms. The mycotoxins include oxalic acid crystals, kojic acid, and cyclic pentapeptides called malformins. The mycotoxins range from moderately to highly toxic in terms of acute toxicity.

A. niger produces oxalic acid and kojic acid abundantly. These two products have only a slight acute toxicity. Oxalic acid has an intraperitoneal LD_{50} of 150 mg/kg in rats and kojic acid has an intraperitoneal LD_{50} of 250 mg/kg in mice (Ueno and Ueno, 1978).

Malformins produced by *A. niger* are more potent toxins, at least by the intraperitoneal route of administration. Malformin

A has an intraperitoneal LD_{50} as low as 3.1 mg/kg in mice (Kobbe et al., 1977.) Pathologic signs accompanying fatality included dilatation with hemorrhage of the gastrointestinal tract and changes in the liver and kidney. Death occurred within four days. In contrast, oral doses up to 50 mg/kg failed to cause evidence of acute toxicity (Yoshizawa, 1975.)

In 1976, Anderegg et al. (1976) reported that a strain of *A. niger* collected from mold-damaged rice produced a highly toxic metabolite, Malformin C, which they established as the disulfide of cyclo-D-cysteinyl-D-cysteinyl-L-valyl-D-leucyl-L-leucyl. When grown on white wheat in a fermentative process, malformin C was highly toxic to newborn rats (LD_{50} 0.9 mg/kg; i.p.) and exhibited antibacterial activity against both gram positive and gram negative bacteria (Ciegler and Vesonder, 1987). Malformin C appears to have more mammalian toxicity than malformin A (Moss, 1977).

Aspergillus niger can interfere with the production of the potent mycotoxin aflatoxin by *A. flavus* under some conditions. Horn and Wicklow (1983) reported that when *A. flavus* and *A. niger* were co-cultured on autoclaved corn kernels, *A. niger* lowered the substrate pH sufficiently to suppress aflatoxin production.

A. niger is able to grow well at high temperatures and has an optimum temperature of 35°C. It germinates at about 77 percent relative humidity (Wyllie and Morehouse, 1971). The production of malformins is related to the composition of the growth substrate and usually occurs in stationary phase cultures. While not always true, mycotoxins are generally produced on solid substrates with high carbon/nitrogen solid content (Ciegler and Kurtzman, 1970; Anderegg et al., 1976). For example, malformins are produced when *A. niger* is grown on onion bulbs (Curtis et al., 1974) and on fermenting grains (Kobbe et al.,1977). A strain of *A. niger* recovered from mold-damaged rice produced Malformin A. A survey to define the number of strains in nature that are Malformin producers appears not to have been made.

The use of radioactively labeled suspected precursors has helped clarify biosynthetic pathways for some mycotoxins. However, the specific enzymes involved in these transformations, their control and genetics are not always known even for well studied mycotoxins such as aflatoxin (Betina, 1989). The loci involved in mycotoxin biosynthesis have not been genetically mapped at present due to the difficulty of working with an asexual microorganism such as *A. niger*.

4. Conclusions

Of the three types of hazard concerns described, two appear to be strain dependent, allergen and toxin production. Because of the number of individuals involved in the few cases of documented occurrence of occupational asthma, the association of the events with *A. niger* fermentations, and the strain specificity involved, proper identification of strains of exempted recipients should limit concern for intrinsic health hazards. Similarly, proper identification of strains should limit concern for mycotoxin production. Opportunistic pathogenicity may be as much a function of the state of the host as of the capability of the infecting fungus, except in the cases of otitis externa where healthy individuals may be infected.

B. Environmental Hazards

1. Hazards to Animals

Livestock ingesting *A. niger* contaminated feed have been shown to be adversely affected by mycotoxins. Calcium depletion and other physiological abnormalities including death can result from ingestion of *A. niger* colonized feed due to the fungal production of oxalic acid or malformins (Austwick, 1965). Chicks and mice were killed after being fed with moldy soybeans and mice died after eating contaminated wheat containing isolates of *A. niger* (Semeniuk, et al., 1971). The cause of death was assumed to be toxicosis, but pathogenicity was observed in some cases. Some of the malformins are currently under development for use as insecticidal compounds (Wicklow, 1991).

2. Hazards to Plants

A. niger has been isolated from 37 genera of plants (Farr et al., 1989). Often these reports involve co-isolation with other perhaps more destructive microorganisms or isolation from a stored plant product. There are reports of *A. niger* being a plant pathogen in peanuts (Jackson, 1962). Apparently, *A. niger* can induce a crown rot of peanuts due to *A. niger*-infected seed under specific hot, humid growth conditions. The mycotoxins described above, namely oxalic acid, malformin A, and malformin C, have been shown to cause significant growth effects such as root curling and top deformation in plants (Anderegg et al., 1976).

A. niger can cause the rotting of numerous fruits, vegetables, and other food products, thus causing substantial economic losses due to spoilage. For example, black rot of onions associated with *A. niger* is responsible for serious losses of onion bulbs in the field and in storage. There are also reports of *A. niger*-induced spoilage of mangos (Prakash and Raoof, 1989), grapes (Sharma and Vir, 1986), and tomatoes (Sinha and Saxena, 1987).

3. Other Ecological Concerns

Members of the *Aspergillus* genus are well known as biodeteriogens (organisms that cause deterioration of materials). For example, *A. niger* causes discoloration and softening of the surface layers of wood, even in the presence of wood preservatives. *A. niger* also causes damage to raw cotton fibers and other cellulose-containing materials, as well as to tanning liquors used in the tanning of hides and leather. It can also attack plastics and polymers such as cellulose nitrate, polyvinyl acetate and polyester-type polyurethanes (Thomas, 1977). *A. niger* is also the major spoilage isolate on bakery products such as English style crumpets (Smith et al., 1988).

4. Conclusions

One set of major concerns for environmental hazard is, like that for human hazard, associated with mycotoxin production. Toxins from *A. niger* may affect other vertebrates and plants as well. Plant pathogenicity, though a possibility for some crops, is not a commonplace concern issue for *A. niger* strains, which are usually considered saprophytic. Because mycotoxin production and crown rot in peanuts may be strain-specific, proper identification of strains would appear to be essential for limitation of ecological, as well as human health, hazard. In addition, *A. niger* is one of many commonplace spoilage-associated fungi, which can cause severe economic effects.

IV. EXPOSURE ASSESSMENT

A. Worker Exposure

Aspergillus niger is considered a Class 1 Containment Agent under the National Institute of Health (NIH) Guidelines for Recombinant DNA Molecules (U.S. Department of Health and Human Services, 1986). In Europe, *Aspergillus* spp. are treated as low-risk-class microorganisms, i.e., category 2 of the European Federation of Biotechnology (Frommer et al., 1989) or category 1 on the OECD containment scale. Category 1 of the European Federation of Biotechnology scale includes organisms deemed harmless, which can be grown under good industrial large scale practices (GILSP), while category 2 organisms like *Aspergillus* require more stringent containment.

No data were available for assessing the release and survival specifically for fermentation facilities using *A. niger*. Therefore, the potential worker exposures and routine releases to the environment from large-scale, conventional fermentation processes were estimated on information available from eight premanufacture notices submitted to EPA under TSCA Section 5 and from published information collected from non-engineered microorganisms (Reilly, 1991). These values are based on reasonable worst-case scenarios and typical ranges or values are given for comparison.

During fermentation processes, worker exposure is possible during laboratory pipetting, inoculation, sampling, harvesting, extraction, processing and decontamination procedures. A typical site employs less than 10 workers/shift and operates 24 hours/day throughout the year. NIOSH has conducted walk-through surveys of several fermentation facilities in the enzyme industry and monitored for microbial air contamination. These particular facilities were not using recombinant microorganisms, but the processes were considered typical of fermentation process technology. Area samples were taken in locations where the potential for worker exposure was considered to be potentially greatest, ie. near the fermentor, the seed fermentor, sampling ports, and separation processes (either filter press or rotary drum filter). The workers with the highest potential average exposures at the three facilities visited were those involved in air sampling. Area samples near the sampling port revealed average airborne concentrations ranging from 350 to 648 cfu/m^3. Typically, the Chemical Engineering Branch would not use area monitoring data to estimate occupational exposure levels since the correlation between area concentrations and worker exposure is highly uncertain. Personal sampling data are not available at the present time. Thus, area sampling data have been the only means of assessing exposures for previous PMN biotechnology submissions. Assuming that 20 samples per day are drawn and that each sample takes up to 5 minutes to collect, the duration of exposure for a single worker will be about 1.5 hours/day. Assuming that the concentration of microorganisms in the worker's breathing zone is equivalent to the levels found in the area sampling, the worst-case daily inhalation exposure is estimated to range up to 650 to 1200 cfu/day. The uncertainty associated with this estimated exposure value is not known (Reilly, 1991).

B. Environmental and General Exposure

1. Fate of the Organism

Aspergilli are among the fungi most frequently isolated from soils and have been found to rapidly colonize and degrade easily available organic matter. For example, in 20 dust samples collected in Saudi Arabia that yielded 22 genera and 46 species when grown on glucose and Czapek's agar, the most common genus was *Aspergillus* and the most common species was *A. niger* (Abdel-Hafez et al., 1985). The abundant asexual spores produced within the conidiophores are resistant to many environmental stresses which enables the organism to survive during inactive periods (Atlas and Bartha, 1981). Thus, it may be assumed that 1) people are frequently exposed to naturally occurring *A. niger.*, and 2) that spores from genetically engineered industrial strains will survive in a similar manner to natural spores upon release into the environment.

This section reviews the potential exposure of populations and the environment outside of a fermentation facility to an industrially used strain of *A. niger*. Because no data are available regarding the ability of industrial strains to disperse and persist in the environment, this assessment is based on mathematical models. These models assume that the microorganisms are dispersed as if they were particles, and that they neither multiply nor die during the dispersal process. These are reasonable assumptions for spores, but they are less appropriate for vegetative forms.

2. Releases

Estimates of the number of *A. niger* organisms released per production batch are tabulated in Table 1. The minimally controlled scenario assumes no treatment of the fermentor off-gas and assumes 100-fold (2 log) reduction of the maximum cell density of the fermentation broth resulting from inactivation (Reilly, 1991). The containment criteria required for the full exemption scenario assume the use of in-line filters to treat vent gases and a 99% removal efficiency under normal operating conditions. They also assume an overall 6-log reduction relative to the maximum cell density of the fermentation broth resulting from inactivation steps (Reilly, 1991).

TABLE 1. Estimated Number of Viable Aspergillus niger Organisms Per Production Batch

Release Media	Minimally Controlled (cfu/day)	Full Exemption (cfu/day)	Release (days/year)
Air Vents	2×10^{8} - 1×10^{11}	2×10^{6} - 1×10^{9}	350
Rotary Drum Filter	250	250	350
Surface Water	7×10^{13}	7×10^{9}	90
Soil/Landfill	7×10^{15}	7×10^{11}	90

Source: Reilly, 1991

3. Air

There are no specific data regarding the survivability of *A. niger* in the atmosphere after release. Human exposure to *A. niger* aerosols, should it occur, would occur via inhalation. Releases from fermentor off-gas may result in nonoccupational inhalation exposures, if the releases are outside of the fermentation facility. To estimate these potential exposures, Versar (1991) used the sector averaging form of the Gaussian algorithm described in Turner (1970) and the release rates estimated by Reilly (1991) and summarized in Table 1. For purposes of this assessment, a release height of three meters and downward contact at a distance of 100 meters were assumed. Under the minimally controlled scenario of no removal of organisms by treatment of off-gasses, ambient human inhalation exposures are estimated to range from 9×10^{6} to 4×10^{9} cfu/day. For systems operating in compliance with criteria required for the full exemption, and thus with 99% reduction of the off-gasses, exposures of 9×10^{4} to 4×10^{7} cfu/day are estimated.

According to Versar (1991), these estimates represent hypothetical exposures under reasonable worst case conditions. However, it should be noted that these exposures are actually substantial overestimates, because industrial fermentors, which may have volumes of 100,000 liters (Finkelstein et al., 1989), themselves are higher than three meters, the assumed stack height [the taller the stack, the lower the predicted organism concentration at 100 meters downwind]. In addition, one of the conditions of the exemption is that the fermentors are not vented directly to the ambient air.

4. Water

Versar (1991) estimated the concentrations of *A. niger* in surface water using stream flow values for water bodies receiving process wastewater discharges from facilities within SIC Code 283 (drugs, medicinal chemicals, and pharmaceuticals). The surface water release data (cfu/day) tabulated in Table 1 were divided by the stream flow values to yield a surface water concentration of the organism (cfu/L). The stream flow values for SIC Code 283 were based on discharger location data retrieved from the Industrial Facilities Dischargers database on December 5, 1991, and surface water flow data retrieved from the RXGAGE database. Flow values were obtained for water bodies receiving wastewater discharges from 154 indirect (facilities that send their waste to a POTW) and direct dischargers (facilities that have an NPDES permit to discharge to surface water). Tenth percentile values indicate flows for smaller rivers within this distribution of 154 receiving water flows and 50th percentile values indicate flows for more average rivers. The flow value expressed as 7Q10 is the lowest flow observed over seven consecutive days during a 10-year observation period. The use of this methodology to estimate concentrations of *A. niger* in surface water assumes that all of the discharged organisms survive wastewater treatment and that growth is not enhanced by any component of the treatment process.

Estimated concentrations of *A. niger* in surface water for minimally controlled and full exemption scenarios are tabulated in Table 2.

TABLE 2. Aspergillus niger Concentrations in Surface Water

Flow	Receiving Stream Flow (MLD*)		Organisms (cfu/l)	
	Mean	Q710	Mean	Q710
Minimally Controlled				
10th Percentile	156	5.60	4.5×10^{4}	1.25×10^{6}
50th Percentile	768	68.13	9.11×10^{3}	1.03×10^{5}
Full Exemption				
10th Percentile	156	5.60	4.5×10^{0}	1.25×10^{2}
50th Percentile	768	68.13	9.11×10^{-1}	1.03×10^{1}

*MLD = million liters per day
Source: Versar, 1991

5. Soil

Because soil is the natural habitat for *A. niger*, long-term survival in soil is expected. Human exposures via dermal contact and ingestion may occur at the solid waste disposal site, if the strain can establish itself. Environmental exposures to terrestrial, avian, and aquatic (via runoff) organisms may also take place. However, it should be noted that for establishment to take place, the introduced *A. niger* will probably have to out compete and displace the indigenous *A. niger* population (Versar, 1991).

6. Summary

Given the ubiquitous nature of *A. niger*, the issue of exposure to a new production strain of this species is basically a question of whether the increase in release of spores due to use of these strains will be noticeable when compared to normal exposure to naturally occurring as well as other commercial production strains. In that context, the number of spores of strains released from production facilities, under the conditions described in Table 2, would not appear to add significantly to the environmental burden of spores produced from natural or other commercial sources.

V. INTEGRATION OF RISK ASSESSMENT

In the previous sections, information regarding the potential exposures and hazards to workers, the general public, animals, plants and the environment was reviewed. This section serves to integrate this information to evaluate the potential risks associated with the industrial use of *Aspergillus niger*.

A. Discussion

1. Risks to Humans and Animals

A. niger has been associated with three categories of adverse human health effects: 1) colonization of spaces in the body and possible invasion of neighboring tissues termed aspergillosis; 2) production of mycotoxins; and, 3) induction of allergic responses such as occupational asthma. The primary hazard to humans and animals appears to be toxicity associated with the production of mycotoxins known as malformins.

Reports associating *A. niger* with infectious diseases in healthy individuals are uncommon, although *A. niger* is a recognized opportunistic pathogen. Given the relative infrequency of anecdotal reports and the frequency with which all humans are exposed to *A. niger*, both by ingestion and inhalation, the probability of colonization in immunocompetent individuals must be quite small. The probability of colonization in immunosuppressed people, however, is relatively high. Nevertheless, given the already ubiquitous presence of *A. niger*, the increased environmental burden of *A. niger* due to release from commercial facilities under conditions imposed by exemption criteria is probably negligible. Thus, it may be concluded that the use of *A. niger* in fermentation facilities is unlikely to increase the baseline risk of infection by *A. niger*.

For the second category of effects, production of toxins, concern is reduced due to available information on the relevant toxins. The higher values of toxicity for Malformins A and C were determined by intraperitoneal injection, a route not considered to be environmentally relevant. Furthermore, data for toxicity via ingestion indicate that the toxicity is much lower by this route (Yoshizawa, 1975). This lower toxicity may be due to the destruction of the malformins, which are cyclic pentapeptides, in the gastrointestinal tract.

A. niger metabolites have caused adverse effects in livestock. The prevalence of strains that can produce these mycotoxins is unknown. Thus, it is uncertain whether the release, via waste disposal or air emissions, of *A. niger* strains capable of producing mycotoxins will add to the environmental burden of mycotoxin producing strains.

Significant environmental release of the mycotoxins themselves is unlikely if the commercial production takes place in a submerged fermentation system, since mycotoxins tend to be elaborated when *A. niger* is grown on solid substrates. However, some production of citric acid does take place on surface cultures, and mycotoxins may be produced. In addition, Malformins are more likely to be produced in cultures at stationary phase, so that production control could limit the elaboration of these toxins. Selection of recipient strains known to be incapable of mycotoxin production or direct toxicity testing of production strains can address the concerns of possible mycotoxin elaboration during commercial production. Inactivation of mycotoxins by physical or chemical means prior to release of either the final product or the fermentation wastes may be another mechanism for reducing risk. It should be noted, however, that methods used to reduce levels of the microbial organism may not inactivate the mycotoxin produced.

Because mycotoxin production is a strain specific phenomenon, the proper identification of strains proposed to be used under conditions of exempt manufacture is critical. The taxonomy of the genus *Aspergillus*, has been stable since 1965, except for proposals that would differentiate among the *A. flavus* group within this genus. However, while its description has not varied, *A. niger* is identified based on physiological and morphological traits that may vary under different conditions of culture. Unless identification of the proposed recipient strains is accomplished by a trained systematic mycologist, who will take care to utilize established identification protocols, or unless strains are obtained from a recognized full service culture collection, misidentification that could lead to use of a production strain capable of exhibiting significant adverse effects, is possible.

The last category of effects, allergen production, may be a serious but isolated concern. Though incidents involving industrial strains associated with occupational asthma in workers are rare, adverse effects have been reported for an industrial use similar to those subject to review under TSCA (Topping et al., 1985). The offending antigen appears to be strain dependent, and the effects noted reinforce the need for proper strain identification, and a documentable history of safe use. Risk may be reduced if a strain has already been used in large scale production without any incidents of occupational asthma, or if strains have been tested for allergenic potential. Additionally, efforts to contain the organism beyond those employed in the above cited example should limit worker exposure and thus risk for occupational asthma.

2. Risks to Plants

Though *A. niger* is generally regarded as a strict saprophyte (Farr et al., 1989, Commonwealth Mycological Institute, 1966), as with effects on animals, the tendency to cause adverse plant effects seem mostly strain specific. An exception may be that *A. niger* appears to be a pathogen to peanuts under some conditions (Jackson, 1962).

Risk would be reduced if waste disposal by landfarming were limited to those strains that have been appropriately tested for plant pathogenicity (for example, against peanuts). In addition, as previously noted, significant environmental release of malformins is unlikely if the commercial production takes place in a submerged system.

3. Other Risks

Other problems with *A. niger* are related to its ability to grow on a variety of substrates, causing deterioration of materials on which it is growing. For example, *A. niger* causes economic losses due to spoilage of bakery, fruit and vegetable products. *A. niger* also damages surface layers of wood, raw cotton fibers and many other materials. However, because *A. niger* is already ubiquitous, the increased environmental burden of *A. niger* due to release from commercial facilities is probably negligible. Thus, the baseline risk of materials damage by *A. niger* will not be affected by the use of *A. niger* in commercial facilities.

4. Summary of Risk Integration

Aspergillus niger is worldwide in distribution and has been isolated from numerous habitats. Humans are continually exposed to *A. niger* spores and vegetative forms on foodstuffs and in the air. Greatest concern for any adverse effects would be for workers exposed to greater spore concentrations rather than the public at large. The vast majority of strains of *A. niger*, especially those used in industrial fermentation, have a history of safe use. While there are sporadic reports to the contrary, most isolates have not been documented to be serious pathogens of humans, animals or plants. Specific strains may produce certain mycotoxins or may elicit serious allergic responses among workers. Those instances of adverse effects seem to be associated with a limited number of strains. With proper characterization of industrial strains, use of those with potential for such effects can be avoided.

B. Recommendation

Recommendations for exempting strains of *Aspergillus niger* should accommodate the strain specific concerns of toxin and allergen production. Those strains having had a documentable stringent determination of strain identification, using experienced personnel and appropriate methods, as well as having a history of safe large-scale industrial use, would need no review to be considered for full exemption listing. Although those strains would qualify as low risk organisms under conditions of production and use, maintenance of documentation that confirms their identity, and the history of safe use is essential.

For industrial strains not meeting the criteria of documented safe use and identification using experienced personnel and appropriate methods a limited review of risks would be needed. The review for listing such strains would focus on the proper identification of the organisms and the likelihood of toxin production under TSCA use conditions.

Strains isolated from nature or having no history of safe industrial scale use could also be considered for exemption provided they have been examined for toxin production and pathogenicity. Laboratory studies using cost-effective methods for determining potential for specific toxin production (e.g., malformins) or pathogenicity (e.g., to peanuts) could be used in lieu of industrial experience. Public comment on this alternative should be sought.

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